| Notice of Allowability | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/851,422 | YU ET AL. | |
| | Examiner | Art Unit | |
| | Karen A Canella | 1642 | |

*-- The MAILING DATE of this communication appears on the cov r sheet with th correspondence address--*

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☐ This communication is responsive to _____.

2. ☒ The allowed claim(s) is/are *2-9, 12–26, renumbered as 1-23 respectively*.

3. ☒ The drawings filed on *03 October 2001* are accepted by the Examiner.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

6. ☐ CORRECTED DRAWINGS ( as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review ( PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   **Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**

7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

**Attachm nt(s)**

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO-1449 or PTO/SB/08), Paper No./Mail Date 1/18/02+2/27/02
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☒ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

Karen A Canella
KAREN A. CANELLA PH.D
PRIMARY EXAMINER

**EXAMINER'S AMENDMENT**

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Kristel Schorr on January 4, 2005.

The Restriction and Species Election Requirements of the Paper mailed April 22, 2002 have been withdrawn.

The application has been amended as follows:

The following list of claims has been substituted for all prior versions and listings of claims:

1. (Canceled)

2. A procytotoxin comprising a peptide comprising at least one lysine residue bound via a peptide bond to at least one amino acid via the ε-amino group of said lysine residue, wherein said peptide without said modification is a cytotoxic peptide, and wherein said at least one amino acid bound via the ε-amino group of said lysine residue acts to prevent the peptide from forming a lytically active conformation, and wherein the cytotoxic peptide is a pore-forming cytolytic peptide.

3. The procytotoxin of claim 2, wherein the pore-forming cytolytic peptide is selected from the group consisting of Ae I, cytolysin of sea anemone, aerolysin, amatoxin, amoebapore, amoebapore homolog from *Entamoeba dispar*, brevinin-1E, brevinin-2E, barbatolysin, cytolysin of *Enterococcus faecalis*, delta hemolysin, diphtheria toxin, El Tor cytolysin of *Vibrio cholerae*, equinatoxin, enterotoxin of *Aeromonas hydrophila*, esculentin, granulysin, haemolysin of *Vibrio parahaemolyticus*, intermedilysin of *Streptococcus intermedius*, the lentivirus lytic peptide, leukotoxin of *Actinobacillus actinomycetemcomitans*,

magainin, melittin, membrane-associated lymphotoxin, Met-enkephalin, neokyotorphin, neokyotorphin fragment 1, neokyotorphin fragment 2, neokyotorphin fragment 3, neokyotorphin fragment 4, NK-lysin, paradaxin, perforin, perfringolysin O, theta-toxin of *Clostridium perfringens*, phallolysin, phallotoxin, and streptolysin.

4. The procytotoxin of claim 3, wherein the cytolytic peptide is an amoebapore

5. The procytotoxin of claim 4, comprising the following structure: Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys(R)-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys(R)-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys(R) (SEQ ID NO: 1), wherein at least one (R) is independently selected from the group consisting of [ε-γ]-Glu, [ε-γ]-Glu-[α-γ]-$(Glu)_{1-3}$, [ε-α]-$(Phe)_{1-3}$, [ε-α]-$(Tyr)_{1-3}$, [ε-α]-$(Trp)_{1-3}$, [ε-α]-$(Lys)_{1-3}$ and [ε-α]-$(Arg)_{1-3}$, wherein [ε-γ] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate, [α-γ] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate, [ε-α] represents a peptide bond between the epsilon amino acid of lysine and the alpha carboxyl group of the indicated amino acid and the subscript indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds.

6. The procytotoxin of claim 3, wherein the cytolytic peptide is a melittin.

7. The procytotoxin of claim 6 consisting essentially of the following structure: Gly-Ile-Gly-Ala-Val-Leu-Lys(R)-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys(R)-Arg-Lys(R)-Arg-Gln-Gln (SEQ ID NO: 2), wherein at least one (R) is independently selected from the group consisting of [ε-γ]-Glu, [ε-γ]-Glu-[α-γ]-$(Glu)_{1-3}$, [ε-α]-$(Phe)_{1-3}$, [ε-α]-$(Tyr)_{1-3}$, [ε-α]-$(Trp)_{1-3}$, [ε-α]-$(Lys)_{1-3}$ and [ε-α]-$(Arg)_{1-3}$, wherein [ε-γ] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate, [α-γ] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate, [ε-α] represents a peptide bond between the epsilon amino group of lysine and the alpha carboxyl group of the indicated amino acid and the subscript

indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds.

8. A procytotoxin comprising a peptide comprising at least one lysine residue bound via a peptide bond to at least one amino acid via the ε-amino group of said lysine residue, wherein said peptide without said modification is a cytotoxic peptide, and wherein said at least one amino acid bound via the ε-amino group of said lysine residue acts to prevent the peptide from forming a lytically active conformation, and wherein said procytotoxin comprises a structure selected from the group consisting of: N-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys([e-g]-Glu-[a-g]-Glu)-$CONH_2$ (SEQ ID NO: 8) and $NH_2$-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys([e-g]-Glu-[a-g]-Glu)-Arg-Lys([e-g]-Glu-[a-g]-Glu)-Arg-Gln-Gln-COOH (SEQ ID NO: 12).

9. (Currently Amended) A pharmaceutical composition, comprising the procytotoxin of claim 2, and a pharmaceutically acceptable excipient.

10. (Canceled)

11. (Canceled)

12. (Currently Amended) The method of claim 13 wherein said cancer cell is selected from the group consisting of prostate, ovarian, lung and skin cells.

13. A method for selectively destroying a target cell that is a cancer cell, comprising contacting the target cell with a procytotoxin, which comprises a peptide comprising at least one lysine residue bound via a peptide bond to at least one amino acid via the ε-amino group of said lysine residue, wherein said peptide without said modification is a cytotoxic peptide, and wherein said at least one amino acid bound via the ε-amino group of said lysine residue acts to prevent the peptide from forming a lytically active conformation, and wherein the cytotoxic peptide is a pore-forming cytolytic peptide.

14. The method of claim 13, wherein the pore-forming cytolytic peptide is selected from the group consisting of Ae I, cytolysin of sea anemone, aerolysin, amatoxin, amoebapore, amoebapore homolog from *Entamoeba dispar*, brevinin-1E, brevinin-2E, barbatolysin, cytolysin of *Enterococcus faecalis*, delta hemolysin, diphtheria toxin, El Tor cytolysin of *Vibrio cholerae*, equinatoxin, enterotoxin of *Aeromonas hydrophila*, esculentin, granulysin, haemolysin of *Vibrio parahaemolyticus*, intermedilysin of *Streptococcus intermedius*, the lentivirus lytic peptide, leukotoxin of *Actinobacillus actinomycetemcomitans*, magainin, melittin, membrane-associated lymphotoxin, Met-enkephalin, neokyotorphin, neokyotorphin fragment 1, neokyotorphin fragment 2, neokyotorphin fragment 3, neokyotorphin fragment 4, NK-lysin, paradaxin, perforin, perfringolysin O, theta-toxin of *Clostridium perfringens*, phallolysin, phallotoxin, and streptolysin.

15. The method of claim 14, wherein the cytolytic peptide is an amoebapore.

16. The method of claim 14, wherein the procytotoxin comprises the following structure: Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys(R)-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys(R)-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys(R) (SEQ ID NO: 1), wherein at least one (R) is independently selected from the group consisting of [e-g]-Glu, [e-g]-Glu-[a-g]-$(Glu)_{1-3}$, [e-a]-$(Phe)_{1-3}$, [e-a]-$(Tyr)_{1-3}$, [e-a]-$(Trp)_{1-3}$, [e-a]-$(Lys)_{1-3}$ and [e-a]-$(Arg)_{1-3}$ , wherein [e-g] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate, [a-g] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate, [e-a] represents a peptide bond between the epsilon amino acid of lysine and the alpha carboxyl group of the indicated amino acid and the subscript indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds.

17. The method of claim 14, wherein the cytolytic peptide is a melittin.

18. The method of claim 17, wherein the procytotoxin consists essentially of the following structure: Gly- Ile-Gly-Ala-Val-Leu-Lys(R)-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-

Ile-Ser-Trp-Ile-Lys(R)-Arg-Lys(R)-Arg-Gln-Gln (SEQ ID NO: 2), wherein at least one (R) is independently selected from the group consisting of-[e-g]-Glu, [e-g]-Glu-[a-g]-$(Glu)_{1-3}$, [e-a]-$(Phe)_{1-3}$, [e-a]-$(Tyr)_{1-3}$, [e-a]-$(Trp)_{1-3}$, [e-a]-$(Lys)_{1-3}$ and [e-a]-$(Arg)_{1-3}$, wherein [e-g] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate, [a-g] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate, [e-a] represents a peptide bond between the epsilon amino group of lysine and the alpha carboxyl group of the indicated amino acid and the subscript indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds.

19. A method for selectively destroying a target cell that is a cancer cell, comprising contacting the target cell with a procytotoxin, which comprises a peptide comprising at least one lysine residue bound via a peptide bond to at least one amino acid via the ε-amino group of said lysine residue, wherein said peptide without said modification is a cytotoxic peptide, and wherein said at least one amino acid bound via the ε-amino group of said lysine residue acts to prevent the peptide from forming a lytically active conformation,-and the procytotoxin comprises the structure $NH_2$-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys([e-g]-Glu-[a-g]-Glu)-Arg-Lys([e-g]-Glu-[a-g]-Glu)-Arg-Gln-Gln-COOH (SEQ ID NO: 12).

20. The procytotoxin of claim 2, wherein the pore-forming cytolytic peptide is selected from the group consisting of Ae I, cytolysin of sea anemone, aerolysin, amatoxin, amoebapore, amoebapore homolog from *Entamoeba dispar*, brevinin-1E, brevinin-2E, barbatolysin, cytolysin of *Enterococcus faecalis*, delta hemolysin, diphtheria toxin, El Tor cytolysin of *Vibrio cholerae*, equinatoxin, enterotoxin of *Aeromonas hydrophila*, esculentin, granulysin, haemolysin of *Vibrio parahaemolyticus*, intermedilysin of *Streptococcus intermedius*, the lentivirus lytic peptide, leukotoxin of *Actinobacillus actinomycetemcomitans*, magainin, melittin, membrane-associated lymphotoxin, Met-enkephalin, neokyotorphin, neokyotorphin fragment 1, neokyotorphin fragment 2, neokyotorphin fragment 3, neokyotorphin

fragment 4, NK-lysin, paradaxin, perforin, perfringolysin O, theta-toxin of *Clostridium perfringens*, phallolysin, phallotoxin, and streptolysin.

21. The procytotoxin of claim 20, wherein the cytolytic peptide is an amoebapore.

22. The procytotoxin of claim 21, comprising the following structure: Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys(R)-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys(R)-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys(R) (SEQ ID NO. 1), wherein at least one (R) is independently selected from the group consisting of [ε-γ]-Glu, [ε-γ]-Glu-[α-γ]-$(Glu)_{1-3}$, [ε-α]-$(Phe)_{1-3}$, [ε-α]]-$(Tyr)_{1-3}$, [ε-α]-$(Trp)_{1-3}$, [ε-α]-$(Lys)_{1-3}$ and [ε-α]-$(Arg)_{1-3}$ , wherein:

[ε-γ] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate,

[α-γ] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate,

[ε-α] represents a peptide bond between the epsilon amino acid of lysine and the alpha carboxyl group of the indicated amino acid, and

the subscript indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds.

23. The procytotoxin of claim 22, wherein R is independently selected from the group consisting of [ε-γ]-Glu and [ε-γ]-Glu-[α-γ]-$(Glu)_{1-3}$, wherein:

[ε-γ] represents a peptide bond between the epsilon amino group of lysine and the gamma carboxyl group of the adjacent glutamate,

[α-γ] represents a peptide bond between the alpha amino group of the first glutamate and the gamma carboxyl group of the second glutamate, and

the subscript indicates that additional numbers of the designated amino acid can be linked to the first via conventional peptide bonds.

24. A method for selectively destroying a target cell that is a cancer cell, comprising contacting the target cell with a procytotoxin, which comprises a peptide comprising at least one lysine residue bound via a peptide bond to at least one amino acid via the ε-amino group of said

lysine residue, wherein said peptide without said modification is a cytotoxic peptide, and wherein said at least one amino acid bound via the ε-amino group of said lysine residue acts to prevent the peptide from forming a lytically active conformation, and the procytotoxin comprises the structure N-Gly-Phe-Ile-Ala-Thr-Leu-Cys-Thr-Lys-Val-Leu-Asp-Phe-Gly-Ile-Asp-Lys-Leu-Ile-Gln-Leu-Ile-Glu-Asp-Lys([ε-γ]-Glu-[α-γ]-Glu)- $CONH_2$ (SEQ ID NO. 8).

25. A procytotoxin comprising a peptide comprising at least one lysine residue bound via a peptide bond to at least one amino acid via the ε-amino group of said lysine residue,

(i) wherein said peptide without the modification is a pore-forming cytolytic peptide,

(ii) wherein said at least one amino acid bound via the ε-amino group of said lysine residue acts to prevent the peptide from forming a lytically active conformation, and

(iii) wherein said cytolytic peptide need not be internalized to cause target-specific cell death.

26. The procytotoxin of claim 25, wherein the pore-forming cytolytic peptide is selected from the group consisting of amoebapore and melittin.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen A Canella whose telephone number is (571)272-0828. The examiner can normally be reached on 10 a.m. to 9 p.m. M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Siew can be reached on (571)272-0787. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Karen A. Canella, Ph.D.
1/4/2005

Karen A. Canella

KAREN A. CANELLA PH.D
PRIMARY EXAMINER